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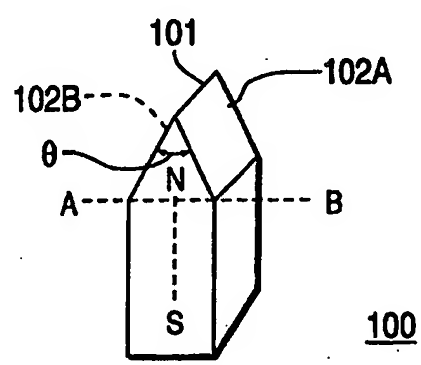
(54) Title: MAGNET

(57) Abstract

A combination for rotating, translocating, or holding a magnetic bead (100) comprising a cell (102A) and a magnet (200) having a pointed end at the north or south pole. Also shown is a magnet comprising two or more magnet slices bonded to each other with alternating orientations of the north and south poles, wherein the junctions between the magnet slices define a surface, whereby local maxima of the magnetic field gradient dH/dx are found at the junctions. Also shown is a method for rotating, translocating, or holding a magnetic bead using the magnets shown.



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MAGNET

This invention was made with U.S. government support under contract No. 70NANB5H1037. The U.S. government has certain rights in this invention.

The present invention relates to the field of magnetic manipulation of objects. More particularly, the present invention relates to novel designs of permanent magnets useful for manipulating magnetic beads, such as paramagnetic beads used for preparing samples for diagnostic assays.

A number of companies are marketing paramagnetic beads that have attached biomolecules, such as antibodies. These companies include Bang Laboratories (Carmel, IN) and Dynal (Lake Success, NY). These beads can be used to bind to specific cell types or specific molecules in a sample, while magnetic properties can be used to retain the beads while non-binding materials are washed away. These beads include those sold under the trade names Dynbeads® M-280, Dynbeads® M-450 and Dynbeads® DNA Direct™ (Dynal, Lake success, NY), the latter of which beads bind nucleic acid and can be used to prepare samples for polymerase chain reaction assays, among other biochemical assays.

These beads can also be used in miniaturized reaction systems for conducting various assays. One shortcoming of such miniaturized systems is that standard magnets when placed against cells of such devices cannot convey sufficient force to the magnetic beads. Accordingly, the potential value of such beads for facilitating biochemical or chemical reactions is lost when used in the context of the aforementioned miniaturized systems.

SUMMARY OF THE INVENTION

It has now been discovered that if one end of a permanent magnet corresponding to either the N r the S pole is appropriat ly shaped, the magnetic field gradient dH/dx near the end f the magnet is

substantially increased, resulting in substantial increase in the force that can be applied to magnetic beads by moving the magnet. Additionally, the magnetic field gradient dH/dx can be increased by constructing a magnet from slices of permanent magnet that are bonded together with alternating N to S pole orientation. The magnetic field gradients in the vicinity of the junction is thereby increased, again resulting in increases in the force that can be applied to magnetic beads.

In particular, the present invention, in one embodiment, pertains to a combination for rotating, translocating, or holding a magnetic bead comprising a cell and a magnet having a pointed end at the N or S pole; wherein the pointed end can be brought in contact with the cell; wherein the pointed end can be brought to within about 250 μm of an interior proximate surface of the cell; wherein the pointed end can be brought to within about 50 μm of the interior proximate surface of the cell; wherein the magnet has a pitched roof shape; wherein the two surfaces forming the roof shape of the magnet have an angular offset of between about 90° and about 150°; and wherein the two surfaces forming the roof share have an angular offset of between about 110° and about 130°. The present invention of this embodiment further comprises the magnetic bead; wherein the magnetic bead is covalently or noncovalently bound to an organic or inorganic molecule; and wherein the bead has a magnetic susceptibility of at least about 0.01 centigrage-gram-second ("cgs") units and the magnet can apply a force of at least about 10 dynes on the bead; wherein the magnet can apply a force of at least about 100 dynes on the bead.

In a second embodiment, the present invention relates to a method of rotating, translocating, or holding a magnetic bead having a magnetic susceptibility of at least about 0.01 cgs units, comprising: (a) using a magnet to create a magnetic field gradient dH/dx along an axis in the plan in which rotating, translocating, or holding of the bead is sought, wherein the field gradient has a magnitude sufficient to cause a

force of at least about 10 dynes on the bead, and (b) moving the magnet to induce corresponding movement in the magnetic bead; wherein the magnetic field gradient dH/dx along an axis in the plane in which rotating, translocating, or holding of the bead is sought has a magnitude sufficient to cause a force of at least about 100 dynes on the bead; wherein the magnetic bead is in a cell; and wherein a chemical or biochemical reaction occurs on the surface of the magnetic bead.

In a third embodiment, the present invention relates to a magnet comprising two or more magnet slices bonded to each other with alternating orientations of the N and S poles, wherein the junctions between the magnet slices define a surface, whereby local maxima of the magnetic field gradient dH/dx are found at the junctions; wherein the magnet slices are between about 0.5 mm and about 2.5 mm in thickness; wherein the magnet comprises at least 4 magnet slices.

In a fourth embodiment, the present invention relates to a combination for rotating, translocating, or holding a magnetic bead comprising a cell and a magnet comprising two or more magnet slices bonded to each other with alternating orientations of the N and S poles, wherein the junctions between the magnet slices define a magnetic surface, whereby local maxima of the magnetic field gradient dH/dx are found at the junctions; wherein the magnetic surface can be brought in contact with the cell to within about 250 μm of the interior proximate surface of the cell.

BRIEF DESCRIPTION OF THE FIGURES

The teachings of the present invention can be readily understood by considering the following detailed description in conjunction with the accompanying figures, in which:

Figure 1 shows a permanent magnet that has a roof shaped at the N pole.

Figure 2 shows a cylindrical magnet that has been formed out of slices of permanent magnets.

Figure 3 shows magnetic beads accumulating at the junctions between slices of permanent magnets.

Figures 4A, 4B and 4C illustrate the use of a magnet of the invention to transport magnetic beads, to hold beads in place while a solution passes over the beads, and to move the beads to provide stirring.

To facilitate understanding, identical reference numerals have been used, where possible, to designate identical elements that are common to the figures.

DETAILED DESCRIPTION

In one embodiment, the present invention relates to a magnet that has the characteristic of effecting a magnetic field of sufficient strength that a magnetic object is manipulated within an enclosed cell when the magnet is placed in contact with an outside wall of the cell. Moreover, in another embodiment, the present invention relates to the combination comprising the aforementioned magnet and a cell, wherein the cell contains reagents and magnetic objects used in the conduct of chemical or biochemical reactions. Such reactions can be for the purpose of diagnostic or forensic assays, preparation of nucleic acids for such assays, or of chemical synthesis of members of a combinatorial library, as is known in the chemical arts, for example.

As noted above, there are difficulties in using magnetic beads within an enclosed cell where it is intended that the magnetic character of the beads is to be used to manipulate certain reagents or effect mixing within the cell. These difficulties can be understood by acknowledging that the force acting on a magnetic bead is proportional to the magnetic field intensity H, the gradient dH/dx of the field, the volume susceptibility κ_v and the volume V of the bead, as shown by the following equation:

$$F = \kappa_v VH\ (dH/dx),$$

wherein volume susceptibility κ_v is the magnetic susceptibility κ_m divided by the volume V of the bead; κ_m is a dimensionless number that is understood in the art and is used herein in the context of the centigrade-gram-second ("cgs") system.

The implication of the aforementioned equation is that the force is proportional to the product of the field intensity, which diminishes with the one over square of the distance from the magnet, and the derivative of the field intensity, which diminishes as one over the cube of the distance from the magnet. Therefore, the force acting on a magnetic bead diminishes with one over the fifth power of the distance from the magnet. The practical implication resulting from this analysis is that it is very difficult to apply sufficient magnetic force to the beads, such that the beads are held in place, translocated, or rotated. Another implication is that standard flat-surfaced magnets, which have small magnetic field gradients at their surfaces, are relatively ineffective for applying force to a magnetic bead.

For the purposes of this application, the term "magnetic bead" will encompass any suitable object, having any suitable shape, preferably with a mass of less than about 0.001 g, that can be pushed or pulled with a magnet. Paramagnetic beads are those that are attracted to the source of a magnetic field when in the presence of such a field of sufficient strength, but otherwise do not display magnetic properties. For example, magnetic beads where, for example, iron, such as Fe_2O_3, is dispersed in a non-magnetic substrate, such as polystyrene, would be considered to be paramagnetic. Preferred paramagnetic beads are made up of a paramagnetic material dispersed in polystyrene and encapsulated within a polymeric film, most preferably a polystyrene film. In some applications, the magnetic beads used are themselves magnetic, and thus are a source of a magnetic field. Such beads comprise magnetic material, such as permanent magnets known in the art. Preferably, the paramagnetic or magnetic material comprises between

about 10% w/w and about 70% w/w of the beads, more preferably between about 30% w/w and about 50% w/w. In a preferred embodiment, the beads have (i) active sites for binding molecules covalently or noncovalently, (ii) bound organic molecules such as biological molecules, or (iii) bound inorganic molecules.

In one embodiment, a magnetic manipulator, comprised of a permanent magnet, is used to attract or repel the aforementioned magnetic beads. The manipulator, and especially the magnet included therewith, is shaped so as to increase the magnitude of the magnetic field gradient at one end. A preferred shape for the magnet is conical, coming to a point at an apex that is placed closest to the beads that are manipulated thereby. Another preferred shape is pyrimidal, again with the pointed apex placed closest to the beads that are manipulated in the context of the present invention. Yet another preferred shape is a pitched roof, wherein again the pointed peak of the roof is placed closest to the the beads to be manipulated. As used herein, the pointed apex of the conical or pyrimidal shapes and the peak of the roof shape, as well as the pointed portions of other suitable shapes, are collectively referred to herein as the "pointed end" of magnets included with the magnetic manipulator used in the context of the present invention. In another embodiment, the magnetic manipulator is comprised of slices of magnets that are bonded together to create junctions where the field gradient magnitudes are larger.

The term "cell" as used herein refers to any structure having an interior cavity suitable for conducting a chemical or biochemical reaction, or transporting reactants or reagents used in such a reaction, including but not limited to channels, microchannels, separation columns, capillaries and reaction or reagent wells. Preferably, the reaction or reagent well has a relatively small volume, such as a volume of about 100 μl or less, such that the magnetic manipulator of the invention when situated in contact with an outside wall of the well can

exert a suitable magnetic field within the well, wherein a suitable magnetic field is one that is able to manipulate a magnetic bead located within the well. A channel, microchannel or capillary through which the aforementioned bead can be transported between wells has dimensions of from about 50 μm to about 500 μm wide and from about 25 μm to about 100 μm deep, but are not limited to these dimensions. Preferred dimensions include those from about 100 μm to about 200 μm wide and from about 30 μm to about 80 μm deep. Such dimensions are determined by consideration of the volumes of reactants required for the chemical or biochemical reaction to be performed, the dimensions of the beads used, whether it is desirable for the beads to leave a reaction or reagent well and enter a channel, microchannel, or capillary, as determined by one skilled in the art.

In Figure 1, magnet 100, which is an embodiment of the magnetic component of a magnetic manipulator, has a roof shape at its N pole with first roof panel 102A and second roof panel 102B. Preferably, first and second roof panels 102A and 102B each have an angular offset θ relative to one another (i.e., a slope) of between about 90° and about 150°, more preferably of between about 110° and about 130°. As illustrated, in one preferred embodiment the apex 101 of the magnet lies in one plane to facilitate bringing a larger sweep of area with a high field gradient adjacent to a cell. Preferably, the apex 101 is smooth. More preferably, the apex 101 has a shape that allows substantially all of the apex (i.e., at least about 90% of the length of the apex) to be placed adjacent to a side of a cell with a flat, tubular or rounded exterior shape.

The magnet 100 is any permanent magnet. Preferably, it is a neodymium-iron-horon magnet, such as is available from the Edmund Scientific C ., Barrington, NJ.

The magnet is most suitably used with a cell constructed of plastic, glass, silica or another material, where the constructi n material

does not have a magnetic property or a thickness that precludes the action of a magnet of the invention on magnetic or paramagnetic beads situated within the cell. The design of the cell should allow substantially all of the apex of a magnet of the invention to approach to within about 250 μm of the interior proximate surface of the cell, preferably to within about 50 μm of the interior proximate surface, wherein the interior proximate surface is the interior surface of the cell that is closest to the magnet. Preferably, the magnet can apply a force on a bead at the interior proximate surface of the cell of at least about 10 dynes, more preferably of at least about 100 dynes, wherein the bead has a magnetic susceptibility κ_M of 0.01 cgs units.

The magnet of the present invention can be used to hold the aforementioned beads in place, to translocate the beads from one position to a second position, or to rotate the beads, wherein the rotation takes place while translocating or holding the beads in place, thus providing for mixing of the beads and the reagent or buffer in which the beads are immersed, or providing a steady state excess of second reactants for reactions that take place upon first reactants that are bound to the beads. Such uses, in particular, include, but are not limited to: (1) translocating the beads from a reagent well to a reaction well, or *vice versa*, (2) removing the beads from a reagent or reaction well, (3) mixing the contents of a reagent or reaction well by rotating the beads, (4) changing the buffer or reagent mix in which the beads are immersed from one buffer or reagent mix to a fresh portion of the same buffer or reagent mix or a different buffer or reagent mix, by holding the beads in place in a fixed or rotating manner in a cell and flowing the fresh or different buffer or reagent mix past the beads.

Figure 2 illustrates an embodiment of a magnet 200 of the present invention with a surface 220 having a first junction 211, second juncti n 212, third junction 213, etc. between first magnet slice 201, second magnet slice 202, third magnet slice 203, etc. Th term

"junction" refers to the point of joinder of the magnet slices along the surface 220. It is the first junction 211, second junction 212, third junction 213, etc. that define areas where the field gradient dH/dx magnitude is greatest. Thus, it is the shape defined by first junction 211, second junction 212, third junction 213, and so on, irrespective of any pits or valleys found between the junctions, that define the shape of surface 220.

The meaning of the phrase "alternating orientations of the N and S poles" can be explained with reference to first junction 211 formed between first slice magnet 201 and second magnet slice 202. If first magnet slice 201 and second magnet slice 202 are separated, the edges of each that formerly formed junction 211 would each be the focus of a local maxima of the magnetic field gradient. The slices are bonded with an "alternating orientation of the N and S poles" if the magnetic field gradient at the junction 211 is greater than the gradient at either such edge of the separated magnet slices.

The magnet slices can be of the same materials that make up the magnet of Figure 1. Bonding between the magnet slices can be accomplished, for instance, using adhesives. Preferably, the magnet slices are bonded together by an epoxy adhesive such as Extra-Fast Setting Epoxy™, available from Hardman, Belleville, NJ.

Preferably, surface 220 is smooth, meaning that it is substantially free of any outcroppings or depressions such that a smooth two dimensional shape can be overlaid without including a gap or separation between adjacent surfaces onto first junction 211, second junction 212, third junction 213, etc. More preferably, the surface 220 has a shape that allows substantially all of the first junction 211, second junction 212, third junction 213, etc. (i.e., at least about 90% of the length of the junctions) to be placed adjacent to a side of a cell of any suitabl shape, which includes without limitations a cell having a flat, tubular or rounded exterior shape.

Preferably, the first magnet slice 211, the second magnet slice 212, the third magnet slice 213, etc., have a thickness of between about 0.5 mm and about 2.5 mm, more preferably between about 1 mm and about 1.5 mm. Preferably, the magnet 200 has at least 4 magnet slices, more preferably at least about 8 magnet slices.

Preferably, magnet 200 is associated with a cell to which substantially all of the first junction 211, second junction 212, third junction 213, etc. can be brought within about 250 μm of the interior proximate surface of the cell, more preferably to within about 50 μm of the interior proximate surface of the cell, wherein the proximate surface is the closest to the position of the magnet.

Figure 3 shows magnetic beads 250 accumulating at the first junction 211, the second junction 212, the third junction 213, etc. Beads also accumulate at the first edge 230A and second edge 230B, since the magnetic field gradient dH/dx is also greater here than it is, for instance, in the space between first junction 211 and second junction 212, although it is not so large at first edge 230A and second edge 230B as it is at first junction 211, second junction 212, third junction 213, and so on.

Figure 4A shows magnet 200 being moved in either of two directions, resulting in the parallel movement of beads 250 situated in cell 300. Figure 4B shows a fluid 310 flowing through cell 300 over magnetic beads 250, where the beads 250 are not carried away by the fluid because they are bound in place by magnet 200. In Figure 4C, magnetic or paramagnetic beads 250 are agitated to provide stirring by rotating magnet 200. The same actions illustrated in Figures 4A - 4C can be carried out using the magnet of the first embodiment, as, for example, illustrated in Figure 1.

<u>IN THE CLAIMS</u>:

1. A combination for rotating, translocating, or holding a magnetic bead comprising a cell and a magnet having a pointed end at the N or S pole.

2. The combination of claim 1, wherein the pointed end can be brought in contact with the cell.

3. The combination of claim 2, wherein the bead has a magnetic susceptibility of at least about 0.01 cgs units and the magnet can apply a force of at least about 10 dynes on the bead.

4. The combination of claim 1, wherein the magnet has a pitched roof shape.

5. A method of rotating, translocating, or holding a magnetic bead having a magnetic susceptibility of at least about 0.01 cgs units, comprising: (a) using a magnet to create a magnetic field gradient dH/dx along an axis in the plane in which rotating, translocating, or holding of the bead is sought, wherein the field gradient has a magnitude sufficient to cause a force of at least about 10 dynes on the bead, and (b) moving the magnet to induce corresponding movement in the magnetic bead.

6. The method of claim 5, wherein the magnetic field gradient dH/dx along an axis in the plane in which rotating, translocating, or holding of the bead is sought has a magnitude sufficient to cause a force of at least about 100 dynes on the bead.

7. The method of claim 6, wherein the magnetic bead is in a cell.

8. A magnet comprising two or more magnet slices bonded to each other with alternating orientations of the N and S poles, wherein the junctions between the magnet slices define a surface, whereby local maxima of the magnetic field gradient dH/dx are found at the junctions.

9. The magnet of claim 8, wherein the magnet slices are betwe n ab ut 0.5 mm and ab ut 2.5 mm in thickness.

10. A combination for rotating, translocating, or holding a magnetic bead comprising a cell and a magnet comprising two or more magnet slices bonded to each other with alternating orientations of the N and S poles, wherein the junctions between the magnet slices define a magnetic surface, whereby local maxima of the magnetic field gradient dH/dx are found at the junctions.

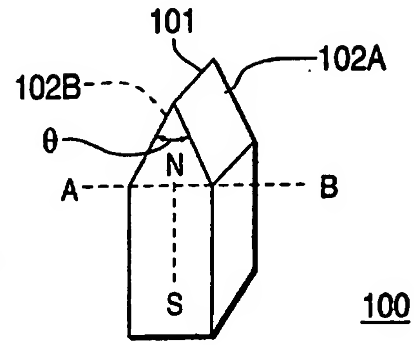

FIG. 1

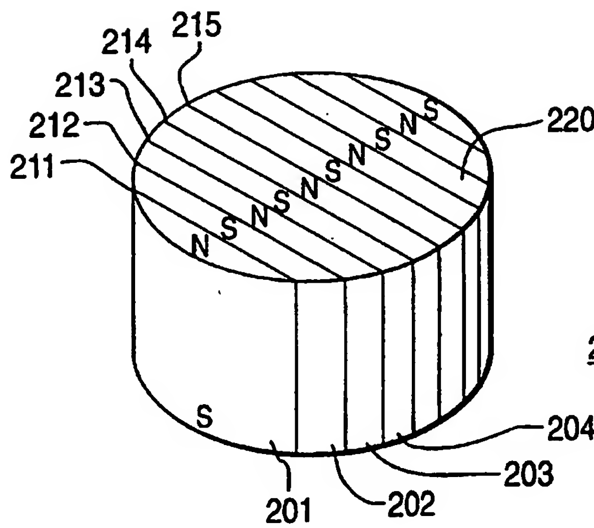

FIG. 2

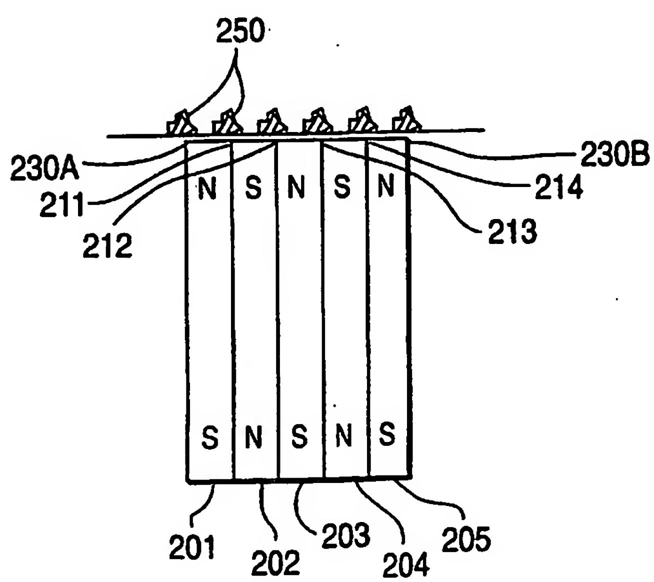

FIG. 3

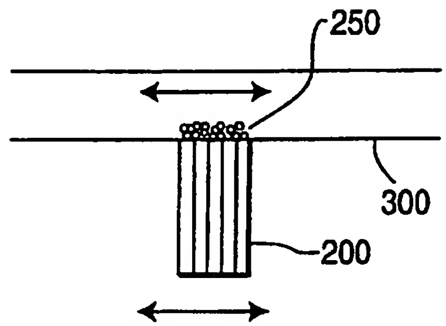

FIG. 4A

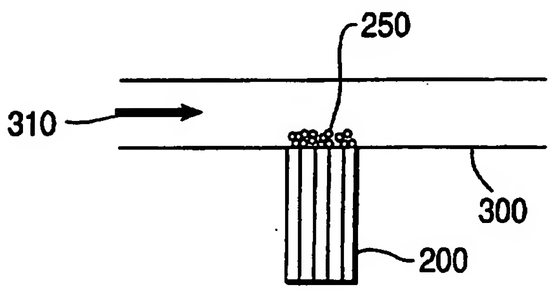

FIG. 4B

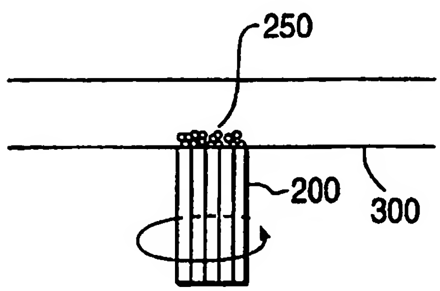

FIG. 4C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/17398

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :HO1F 7/02, 3/00
US CL : 335/302,303,304,305,306
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 335/302,303,304,305,306

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

NONE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,387,893 A (OGURIYAMA et al.) 07 FEBRUARY 1995, see entire document.	1-10
Y	US 4,998,083 A (ABRLE) 05 MARCH 1991, see entire document.	1-10
Y	US 5,237,301 A (UNKELBACK et al.) 17 AUGUST 1993, see entire document.	1-10

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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